P-ISSN: 2349-8528

E-ISSN: 2321-4902

www.chemijournal.com

IJCS 2020; 8(5): 420-422

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Received: 15-06-2020

Accepted: 18-08-2020

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Detection of FFA content of coconut milk by using screen-printed electrode

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DOI: <https://doi.org/10.22271/chemi.2020.v8.i5f.10330>

Abstract

Coconut milk is an aqueous solution which is extracted from coconut meat from pressing or squeezing. The quality attributes of coconut milk are profoundly affected by many factors, and coconut milk is highly susceptible to chemical and biochemical spoilage like lipid oxidation. The quality of coconut milk is usually found out by FFA (% of lauric acid) content in it, but it requires a lot of solvents and complicated procedure. The screen-printed electrode is made of flexible or rigid substances that can be printed on a flexible PET film or substances. Screen-printed electrode biosensor was used to analyse the Triglyceride content and to correlate with FFA content to check the quality of coconut milk. The triglyceride content and FFA content was analysed for the 5-hour interval for two days and found that FFA content was increasing from 0.448 to 1.455 at the end of 45 hours and triglyceride content was decreasing from 1.18 to 0.36mM. The validation of the developed sensor was showing good results with the UHPLC method with a coefficient of determination ($R^2=0.96$). The performance valuation of the developed biosensor was also evaluated.

Keywords: Coconut, triglyceride, quality, electrochemical

1. Introduction

Coconut (*Cocos nucifera* L.) is a monocotyledon palm family of Palmaceae (Ohler, 1999). *Cocos nucifera* L. is generally referred to as a "tree of life" and is one of the most useful trees in the world. The edible part of the coconut can be used for the manufacture of a variety of commercial products including dried coconut (copra), coconut milk, desiccated coconut, coconut flour, coconut oil, coconut vinegar, virgin coconut oil and a few more (Tecson-Mendoza., 2007; Naik *et al.*, 2015) ^[10, 6].

Coconut milk is oil in water emulsion which water acts as continuous phase and oil acts as a dispersed phase. Coconut milk is usually extracted by squeezing or pressing the grated coconut meat in the home by adding with or without the addition of water to it, whereas as in commercial or industrial scale employs hydraulic or screw press to extract the coconut milk (Tansakul & Chaisawang, 2006) ^[9]. Coconut milk is rich in sugar, minerals, vitamins, protein and amino acid compounds. Coconut milk which contains 3.8% of protein and 35.2% of fat content in it (Raghavendra & Ksms, 2010; Ariyaprakai & Tananuwong, 2015) ^[8, 1]. Coconut milk has been used as an ingredient for several cuisines such as desserts and curries. Besides impotence from food ingredient, coconut milk is also used for the manufacture of virgin coconut oil (VCO). Nowadays, coconut milk has become an impotent food for both cooking material and emulations due to its rich in nutrients value and flavour (Iguttia *et al.*, 2011) ^[3].

The quality attributes of coconut milk are profoundly affected by many factors, and coconut milk is highly susceptible to chemical and biochemical spoilage like lipid oxidation due to its high oil content. For coconut milk, fat content is the critical factor for any categorisation. According to CODEX STAND, 240-2003 coconut milk should contain at least 10% of fat, 2.8% of non-fat solid and 12.7 -25.3% of total solid (Pak 2016) ^[7]. The chemical deterioration occurs by lipolysis and lipid oxidation in saturated and unsaturated fatty acids which results in objectionable taste and flavour. The hydrolysis of triglycerides content can be particularly rapid when it is catalysed by lipase enzyme. The coconut milk contains medium chain triglycerides of saturated fat, and these triglycerides are susceptible to hydrolysis into free fatty acids (FFA). The milk fat can also be degraded by oxidation process chemically, the attack on double bonds of triglycerides by oxygen.

The oxidation of the unsaturated phospholipids in milk produces off flavour (Cui & Decker, 2016) [2].

The quality of coconut milk is usually found out by FFA (% of lauric acid) content in it. The FFA content is usually done by titration method, which requires many solvents, and it is also challenging to find the endpoint of the titration. Other analytical techniques like high-performance liquid chromatography, spectrophotometric method, near-infrared and gas chromatography techniques require highly skilled labour, too much expensive and need lots of solvent preparation.

In order to overcome these disadvantages, screen printed technology can be used to find the quality of coconut milk at regular interval of time, and any places can be used. The screen-printed electrode is made of flexible or rigid substances that can be printed on a flexible PET film or substances. The screen printed electrodes (SPE) has high sensitivity, selectivity and cost of analysis can also be reduced compared to other techniques (Thiyagarajan *et al.*, 2014) [11]. The screen-printed works on electrochemical analysis, such as amperometric detection which is gaining importance as it uses a very less amount of solvents and can be analysed quickly in all food products (Jadav *et al.*, 2016).

So this study involves a novel method of SPE biosensor was used to analyse the Triglyceride content and to correlate with FFA content to check the quality of coconut milk.

2. Materials and Methods

2.1 Reagents and Materials

The matured coconuts free from damage were purchased from the local market in Thanjavur district, Tamil Nadu, for the extraction of fresh coconut milk. The reagents triton X100, potassium hydroxide, ethanol, phenolphthalein was purchased from ponmmmani chemicals, Trichy, India.

2.2 Development of SPE

The SPE was developed according to (Manoj *et al.*, 2020) [5]. The SPE developed was made of three electrodes with the working electrode as carbon, reference electrode as Ag/Ag Cl and counter electrode as carbon. An amperometric enzyme-based SPE was developed for finding the amount of triglyceride content in coconut milk. Lipase, GK and GPO were taken in 10:5:2 enzyme ratio and being immobilised into a 45mg concentration of gelatin membrane solutions. 40 μ L of enzyme mixture, which contains lipase, GK and GPO were mixed with 10 μ L of gelatin solution was immobilised to a working electrode made of carbon and kept at four °C for 30 min. After immobilisation, then a 2.5% glutaraldehyde solution was added to the working electrode for 5min. The developed biosensor was taken into analysis if triglyceride content in coconut milk.

The experiment was conducted at pH 7.0 and buffer concentration of the 100mM solution. The developed SPE biosensor was taken for measurements which are connected to a potentiostat for measurements of current. The experiments were carried out at room temperature, and the current was measured for different triolein concentration by keeping a single drop on the working electrode surface area. Then the triglyceride content was measured by correlating current produces with the standard triolein solution measured. By this way, we can analyse the total amount of triglyceride presents in coconut milk (Manoj *et al.*, 2020) [5].

2.3 The free fatty acid content

An accurately measured 2mL of sample is taken in a 250 mL conical flask, and 20 mL of freshly neutralised ethanol is added to it. Furthermore, about 2-3 drops of phenolphthalein indicator are added to the solution. The sample is titrated against 0.1N KOH solution shaking vigorously during the titration until pale pink colour is obtained (AOAC method 922.02 2002).

2.4 Validation and performance evaluation of developed biosensor

To study the accuracy of the present method, the triglyceride content was measured from the ultra-high-performance liquid chromatography (UHPLC) method for coconut milk with different storage period, and then it was correlated with the developed biosensor method. The performance evaluation like reproducibility, thermal stability and storage stability of SPE biosensor was evaluated.

3. Results and Discussion

3.1 Detection of triglyceride from coconut milk

The SPE, which is immobilised with enzymes, was used to detect the amount of triglyceride in coconut milk. The 5mL of coconut milk was mixed with the 10 mL buffer solution (0.1M of sodium phosphate) by adding 1mL of 5% of Triton X100 solution as an emulsifying agent. Then a single drop of solution was taken and kept on working electrode in SPE connected with potentiostat for measurement of output current. The current values of the analysed are shown in figure 1. The current measured was incorporated using empirical relationship $y = 88.4x + 73.53$ to know the amount of triglyceride content (Manoj *et al.*, 2020) [5]. The output current was measured for every 5-hour interval till 45-hour time. The triglyceride content measured in given in table 1.

Fig 1: The relationship between current produced with time (Manoj *et al.*, 2020) [5]

3.2 Detection of quality of coconut milk

Then FFA content (% lauric acid) was measured titration of a sample against KOH till the colour of solution changes to pale pink. The reading was taken for the 5-hour interval for two days and found that FFA content was increasing from 0.448 to 1.455 at the end of 45 hours and triglyceride content was decreasing from 1.18 to 0.36mM, which is shown in table 1.

Table 1: Relationship between triglyceride content and FFA content of a coconut milk

Hour	Triglyceride content (mM)	% of lauric acid
5	1.18	0.448
10	0.852	0.591
15	0.638	0.677
20	0.548	0.724
25	0.457	0.811
30	0.408	0.875
35	0.359	0.936
40	0.318	1.099
45	0.236	1.455

3.3 Validation of developed biosensor

The validation of the developed biosensor was found out by comparing with the UHPLC method for triglyceride determination in coconut milk with different storage period. The accuracy of developed biosensor found a good correlation of $r=0.97$

Table 2: Correlation between developed biosensor method and standard UHPLC method

S. No	Triglyceride content		Standard Deviation
	HPLC(Mm)	Biosensor (Mm)	
1	1.36	1.08	0.14
2	0.81	0.79	0.01
3	0.61	0.5	0.055

3.4 Performance evaluation of SPE

3.4.1 Reproducibility

The ability of the SPE to give the same value if we test for the same coconut milk was tested. Moreover, it was found for 0.5mM triglyceride content in coconut milk average values is found to be 0.56Mm and standard deviation of as 0.083×10^{-3} Mm ($n=3$).

3.4.2 Thermal stability of the SPE

The working electrode was kept in an incubator maintained at 40 °C for 4 hours, and experiments were performed every 1 hour. The biosensor showed a response of 72%, 48%, 26%, and 12% activity respectively at 40 °C. At high-temperature enzymes are denatured and resulted in a reduction of activities

3.4.3 Storage stability of the SPE

The storage stability of the working electrode was performed by keeping it at refrigerated conditions. The drawback of the SPE system was that it could be measured three times using one SPE. So for two days' interval, it was stored up to 6 days, and the biosensor response was noted. The biosensor response for 2th, 4th and 6th days were found to be 85.26%, 73.21% and 54.33%, respectively.

4. Conclusion

A novel method for detection of FFA content in coconut milk was evaluated using an SPE. The developed SPE, which was used to detect triglyceride content, was used to evaluate the quality of coconut milk. The triglyceride content was analysed from the SPE, in which enzymes were already immobilised, and a drop of the sample was used for analysis. The current obtained was correlated with the empirical equation, and we will know the triglyceride content is a sample. Then the FFA content was measured at 5-hour interval for two days and correlated with triglyceride content. In future, we can able to detect the quality of coconut milk that it is spoiled or not by using simple SPE technology.

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